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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER
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RAO, MANJUNATH N

ART UNIT	PAPER NUMBER
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1652

DATE MAILED: 11/05/2002

19

Please find below and/or attached an Office communication concerning this application or proceeding.

**Office Action Summary**

Application No.

09/246,451

Applicant(s)

ARNOLD ET AL.

Examiner

Manjunath N. Rao, Ph.D.

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-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

**P riod for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

- 1) ☒ Responsive to communication(s) filed on 20 August 2002.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

- 4) ☒ Claim(s) 146-189 is/are pending in the application.
- 4a) Of the above claim(s) \_\_\_\_\_ is/are withdrawn from consideration.
- 5) ☒ Claim(s) 146-155, 160 and 167-173 is/are allowed.
- 6) ☒ Claim(s) 156-159, 161-166, 176, 177, 180, 181, 184, 185, 188 and 189 is/are rejected.
- 7) ☒ Claim(s) 174, 175, 178, 179, 182, 183, 186 and 187 is/are objected to.
- 8) ☐ Claim(s) \_\_\_\_\_ are subject to restriction and/or election requirement.

**Application Papers**

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☒ The drawing(s) filed on 05 November 2001 is/are: a) ☒ accepted or b) ☐ objected to by the Examiner.
- Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
- 11) ☐ The proposed drawing correction filed on \_\_\_\_\_ is: a) ☐ approved b) ☐ disapproved by the Examiner.
- If approved, corrected drawings are required in reply to this Office action.
- 12) ☐ The oath or declaration is objected to by the Examiner.

**Priority under 35 U.S.C. §§ 119 and 120**

- 13) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some \* c) ☐ None of:
- ☐ Certified copies of the priority documents have been received.
  - ☐ Certified copies of the priority documents have been received in Application No. \_\_\_\_\_.
  - ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).
- \* See the attached detailed Office action for a list of the certified copies not received.
- 14) ☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).
- a) ☐ The translation of the foreign language provisional application has been received.
- 15) ☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

**Attachment(s)**

- 1) ☒ Notice of References Cited (PTO-892) 4) ☐ Interview Summary (PTO-413) Paper No(s). \_\_\_\_\_
- 2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948) 5) ☐ Notice of Informal Patent Application (PTO-152)
- 3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) 10, 15. 6) ☐ Other: \_\_\_\_\_

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### **DETAILED ACTION**

#### **CONTINUED EXAMINATION UNDER 37 CFR 1.114 AFTER FINAL REJECTION**

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 8-20-02, paper No. 17 and 18 has been entered.

Claims 146-189 are currently pending in this application.

Applicants' amendments and arguments filed on 8-20-02, paper No. 18, have been fully considered and are deemed to be persuasive to overcome some of the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

#### ***Claim Rejections - 35 USC § 112***

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 156-159, 176, 177, 180, 181, 184, 185, 188 and 189 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

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Claims 156-159, 176, 177, 180, 181, 184, 185, 188 and 189 are directed to polypeptide cytochrome P450<sub>cam</sub> oxygenase variants which are at least two to ten times as active and/or as stable as the wild type cytochrome P450<sub>cam</sub> and have at least 90% sequence identity to SEQ ID NO:2. Claims 156-159, 176, 177, 180, 181, 184, 185, 188 and 189 are rejected under this section of 35 USC 112 because the claims are directed to a genus of polypeptides including modified polypeptide sequences, modified by at least one of deletion, addition, insertion and substitution of an amino acid residue in SEQ ID NO:2 and fragments of SEQ ID NO:2 that have not been disclosed in the specification. No description has been provided of all the modified polypeptide sequences encompassed by the claim. No information, beyond the characterization of three specific mutants with changes to amino acids at three specific positions in SEQ ID NO:2 has been provided by applicants which would indicate that they had possession of all the claimed genus of modified polypeptides. The specification does not contain any disclosure of the structure and function of all the polypeptide sequences which are 90% identical to SEQ ID NO:2 and have *at least* 2 or 10 times (specifically applicants have not described the structure of polypeptides which have more than 10 times the activity and stability of the wild type enzyme which are encompassed in the above claims) the activity and stability when compared with the wild type enzyme, including fragments and variants within the scope of the claimed genus. The genus of polypeptides claimed is a large variable genus including peptides which can have a wide variety of structures and with the potentiality of different levels of activity and stability. Therefore many structurally and functionally unrelated polypeptides are encompassed within the scope of these claims. The specification discloses only three specific variant species of the claimed genus which is insufficient to put one of skill in the art in possession of the attributes

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and features of all species within the claimed genus. Therefore, one skilled in the art cannot reasonably conclude that applicant had possession of the claimed invention at the time the instant application was filed.

Applicant is referred to the revised guidelines concerning compliance with the written description requirement of U.S.C. 112, first paragraph, published in the Official Gazette and also available at [www.uspto.gov](http://www.uspto.gov).

In response to the previous Office action, applicants have traversed the above rejection arguing that Examiner's contention is incorrect and that the specification provides a multitude of cytochrome P450 variants having improved oxygenase activity. Applicants also provide examples 19 and 20 in support of their argument which they argue shows a *vast number* of mutants *many of which have at least twice the activity* of P450<sub>cam</sub> and the further characterization of three mutants. Examiner respectfully disagrees that his contention is incorrect. Examiner also disagrees with the applicants that providing examples 19 and 20 which shows "a *vast number* of mutants *many of which have at least twice the activity* of P450<sub>cam</sub>" to be persuasive to overcome the above rejection. While Examiner acknowledges that applicants have in deed provided "examples", such examples still do not describe all the mutants that they are claiming and are encompassed in the above claims. Examiner would like to draw the attention of the applicants to the claim language wherein claims are drawn to variants which are not only 90% identical to SEQ ID NO:2 but also have "*at least two times*" or "*at least 10 times*" the activity/stability of wild-type P450<sub>cam</sub>. The above claims now are not just limited to enzymes which have 90% identity to SEQ ID NO:2 but also to those enzymes which in addition have improved activity/stability ranging from 2 to 10 or 100 or even 1000 times the

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activity/stability of the wild-type. Just because applicants have now introduced a “per cent identity” limitation, it does not mean that applicants are simply claiming variants which are 2 times or 10 times more active/stable than wild type. Therefore applicants have not described all those variants which are 90% identical in amino acid sequence to SEQ ID NO:2 and “at least two times” or “at least 10 times” active/stable when compared to wild-type P450<sub>cam</sub>.

Applicants also quote from the official guidelines and discuss an example taken from the guidelines. However, that example from the guidelines is not applicable to the instant case since pending claims are not similar to those in the example.

As discussed in the written description guidelines, the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus. A representative number of species means that the species which are adequately described are representative of the entire genus. **Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus.** Satisfactory disclosure of a representative number depends on whether one of skill in the art would recognize that the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed. For inventions in an unpredictable art, adequate written description of a genus which embraces widely variant species cannot be achieved by disclosing

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only one species within the genus. In the instant case the claimed genera of claims 156-159, 176, 177, 180, 181, 184, 185, 188 and 189 includes species which are widely variant in function (i.e., those which are *at least* 2 times (meaning 2 or any number more than 2) or *at least* 10 times (meaning 10 or any number more than 10) active/stable when compared to wild type). As such, neither the description of the structure of SEQ ID NO:2 or polypeptide which are 90% identical to SEQ ID NO:2 with oxygenase activity is sufficient to be representative of the attributes and features of the entire genus.

### ***Claim Rejections - 35 USC § 102***

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

Claims 156, 176, 177 are rejected under 35 U.S.C. 102(a) as being anticipated by England et al. (FEBS Letts, 1998, Vol. 424:271-274 provided in the IDS). This rejection is based upon the public availability of a printed publication. Claims 156, 176, 177 of the instant application is drawn to a cytochrome P450 mutant having a catalytic activity at least two times the catalytic activity of a wild type cytochrome P450<sub>cam</sub> oxygenase of *Ps.putida* in promoting oxygenation of an oxygenase substrate in the presence of an oxygen donor and at least 90% sequence identity to SEQ ID NO:2. England et al. disclose an identical enzyme, a mutant of cytochrome P450<sub>cam</sub> isolated from *Ps.putida* which exhibits oxidation of naphthalene and

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pyrene with rates of one to two orders of magnitude faster than the wild type enzyme. Thus England et al. anticipate claims 156, 176, 177 of this application as written.

***Claim Rejections - 35 USC § 103***

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 156-159, 161-166, 176-177, 180-181, 184-185, 188-189 are rejected under 35

U.S.C. 103(a) as being unpatentable over Kuchner et al. (TIB Tech, 1997, Vol. 15:523-530),

England et al. (FEBS Letts, 1998, Vol. 424:271-274 provided in the IDS) and Wong et al.. (WO

97/16553, 5-9-1997). Claims 156-159, 161-166, 176-177, 180-181, 184-185, 188-189 in this

instant application are drawn to a cytochrome P450 mutant having a catalytic activity at least

two times to ten times the catalytic activity and stability of a wild type cytochrome P450<sub>cam</sub>

oxygenase of *Ps.putida* in promoting oxygenation of an oxygenase substrate in the presence of

an oxygen donor and at least 90% sequence identity to SEQ ID NO:2 and wherein the oxygenase

variant was identified by a method comprising contacting the test enzyme variant with an

oxygenated substrate and the oxygen donor under conditions allowing the formation of an

oxygenated product wherein said enzyme variant is an oxygenase enzyme, providing a coupling

enzyme capable of promoting the formation of a detectable composition from the oxygenated

product, detecting the detectable composition and selecting any test enzyme having at least 2-10

times the catalytic activity of the wild type oxygenase enzyme in the presence of the oxygen



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donor, wherein the detecting the composition comprises detection of at least one of the ultraviolet light, color change, fluorescence and luminescence, wherein the organic substrate is selected from a group consisting of a compound like naphthalene and oxygen donor is selected from a group wherein one such donor is  $H_2O_2$  and the coupling enzyme is selected from a group of enzymes wherein HRP is one of the enzyme.

Kuchner et al. teach a powerful alternative to rational approaches for engineering biocatalysts which is called as directed evolution of enzyme catalysts. The reference teaches in general, methods to evolve an enzyme to improve its characteristics and asserts that prerequisites for such directed evolution are functional expression in a suitable microbial host, a rapid screen for the desired feature(s) and a well-thought-out working strategy for navigating protein landscapes. The reference teaches *in vitro* techniques such as creating and searching combinatorial enzyme libraries as well as successful examples of different evolutionary strategies being used. However, the reference does not teach the evolution of *Ps.putida* cytochrome P450<sub>cam</sub>.

England et al. teach a variant in which a single amino acid in a wild type cytochrome P450<sub>cam</sub> has been mutated leading to a mutant which oxidizes naphthalene and pyrene with rates of one to two orders of magnitude faster than the wild type enzyme. The reference teaches methods to obtain such a mutant and assay for the oxidizing activity.

Wong et al. teach a mutant cytochrome P450<sub>cam</sub> in which a single amino acid has been mutated with the aim of redesigning the wild type *Ps.putida* P450<sub>cam</sub>, such that it is able to more effectively carry out specific oxidations of organic molecules whether or not these are substrates for the wild-type protein. The reference also goes on to teach other specific mutations

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(i.e., changes at specific amino acids) that have already been done to the above enzyme and discusses the roles of specific amino acids.

Thus there appears to be a conscientious effort in the art to evolve this enzyme in order to make it more active and at the same time more stable.

With the above three references in hand it would have been obvious to one of ordinary skill in the art to plan and evolve the above enzyme such that it is at least two to ten times more active and stable than the wild type enzyme. Based on the above teachings, it would have been obvious to one of ordinary skill in the art to mutate as many as 1 or more amino acids and come up with a variant which is at least 2-10 times active and stable than the wild type enzyme. One of ordinary skill in the art would have been motivated to do so because it is well known in the art that this specific enzyme has the capabilities to detoxify certain commonly used organic compounds such as naphthalene. One of ordinary skill in the art would have a reasonable expectation of success since England et al. and Wong et al. provide techniques to develop the mutants and also demonstrate mutants they developed --which are more efficient than wild type enzyme-- using such techniques.

Therefore the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later

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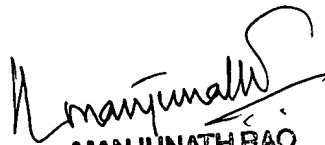
invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

***Conclusion***

Claims 146-155, 160, 167-173 are allowable.

Claims 174-175, 178-179, 182-183, 186-187 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Manjunath Rao whose telephone number is (703) 306-5681. The Examiner can normally be reached on M-F from 7:30 a.m. to 4:00 p.m. If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, P.Achutamurthy, can be reached on (703) 308-3804. The fax number for Official Papers to Technology Center 1600 is (703) 305-3014. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

  
MANJUNATH RAO  
PATENT EXAMINER

Manjunath N. Rao Ph.D.  
11/1/02